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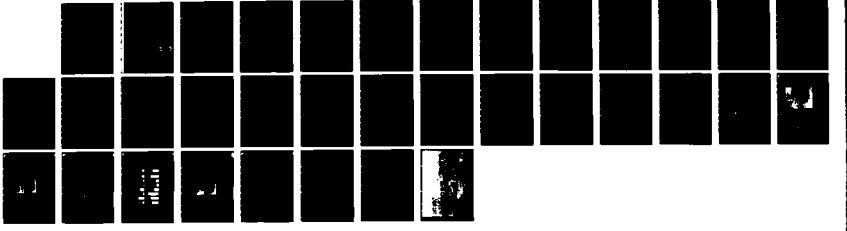

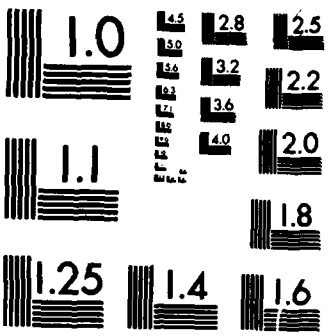

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Between 4 and 10 weeks of age 10 normally reared kittens were bilaterally implanted with osmotic minipumps. The visual cortex of one cortical hemisphere was infused with 4 mM 6-hydroxydopamine while the other hemisphere received only a vehicle solution. The pumps delivered the solutions at 1 μl/hr for one week concurrent with monocular deprivation. Subsequent electrophysiological recording was performed blind and revealed a marked effect of the 6-OHDA treatment: while most cells in the control hemisphere were primarily activated by stimulation of the non-deprived eye, cells in the 6-OHDA-

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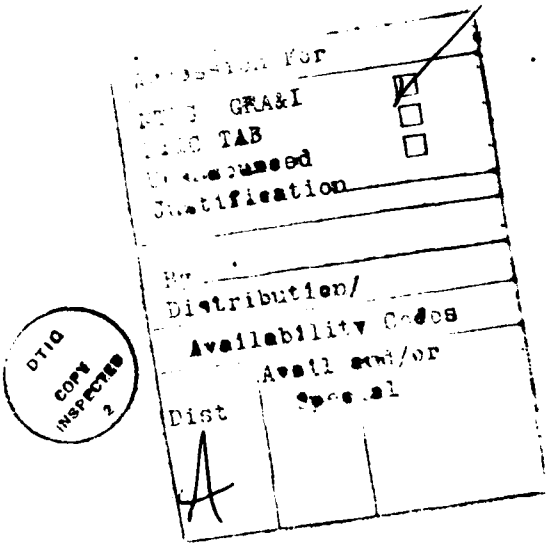
treated hemisphere were significantly more binocular. High pressure liquid chromatography revealed that the loss of normal ocular dominance plasticity in 6-OHDA-infused hemispheres was always accompanied by at least a 50% decrease in cortical norepinephrine levels and a smaller decrease in dopamine levels. Furthermore, there appeared to be a positive correlation of the degree of ocular dominance shift and the relative amount of norepinephrine present. These results are consistent with the hypothesis that catecholamines, especially norepinephrine, are normally required for ocular dominance plasticity during the critical period in kittens.



Effects of Intracortical Infusion of 6-Hydroxydopamine on the Response of Kitten Visual Cortex to Monocular Deprivation

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Summary

Between 4 and 10 weeks of age 10 normally reared kittens were bilaterally implanted with osmotic minipumps. The visual cortex of one cortical hemisphere was infused with 4 mM 6-hydroxydopamine while the other hemisphere received only a vehicle solution. The pumps delivered the solutions at 1 µl/hr for one week concurrent with monocular deprivation. Subsequent electrophysiological recording was performed blind and revealed a marked effect of the 6-OHDA treatment: while most cells in the control hemisphere were primarily activated by stimulation of the non-deprived eye, cells in the 6-OHDA-treated hemisphere were significantly more binocular. High pressure liquid chromatography revealed that the loss of normal ocular dominance plasticity in 6-OHDA-infused hemispheres was always accompanied by at least a 50% decrease in cortical norepinephrine levels and a smaller decrease in dopamine levels. Furthermore, there appeared to be a positive correlation of the degree of ocular dominance shift and the relative amount of norepinephrine present. These results are consistent with the hypothesis that catecholamines, especially norepinephrine, are normally required for ocular dominance plasticity during the critical period in kittens.

Key words: Visual cortex - Plasticity - Catecholamines - 6-Hydroxydopamine - Cat

Introduction

In recent years it has been suggested that catecholamines (CA) are required for neuronal plasticity in the neocortex (Crow 1968; Kasamatsu and Pettigrew 1976; Kety 1970). The most thorough test of this hypothesis has been made in a series of experiments performed by Kasamatsu and Pettigrew (Kasamatsu and Pettigrew 1976, 1979; Kasamatsu et al. 1979, 1981B; Pettigrew and Kasamatsu 1978) who used the monocular deprivation paradigm with kitten visual cortex as a test system. In their control kittens they found the usual effect of monocular deprivation during the critical period--within a week the majority of cells lost their normal binocular responsiveness and could be driven only by stimulation of the non-deprived eye. But, in animals given the neurotoxin 6-hydroxydopamine (6-OHDA) to deplete cortical CAs, the ocular dominance shift failed to occur and cells remained binocularly driven. In their most incisive experiments Kasamatsu and Pettigrew pioneered the use of miniature osmotic pumps to infuse 6-OHDA continuously to local regions of cortex in one hemisphere while they used the other hemisphere as a control (Kasamatsu et al. 1979, 1981B; Pettigrew and Kasamatsu 1978). Following monocular deprivation, normal plasticity was again disrupted in CA depleted cortex as indicated by the lack of shift in ocular dominance of visual cells. Because both noradrenergic (Itakura et al. 1981) and dopaminergic (Tork et al. 1979; Tork and Turner 1981) fibers project to visual cortex, Kasamatsu et al. (1979) also used the minipumps to add norepinephrine (NE) to the cortex previously depleted of CAs to demonstrate that NE, specifically, is necessary for plastic change to occur. The balance of the evidence suggests that catecholamines, especially NE, are necessary for the cortical changes observed in kittens which have restricted vision during the critical period.

We attempted to extend these findings by using another method of cortical CA depletion--neonatal i.p. injection of 6-OHDA. In very young rodents this treatment allows 6-OHDA to cross the immature blood-brain barrier and create a

long lasting CA deficit, especially in the neocortex (Clark et al. 1972; Sachs 1973; Sachs and Jonsson 1975). We succeeded in showing that this technique is also effective for CA depletion in kitten cortex, but we found no loss of plastic response to monocular deprivation, even in kittens with less than 5% of normal NE levels in the neocortex (Bear et al. 1983; Bear and Daniels 1983). Puzzled by this result we decided to reassess the changes in cortical plasticity and CA levels caused by minipump infusion of 6-OHDA into area 17. We incorporated two changes from the methodology which Kasamatsu and Pettigrew used in a similar experiment: the minipumps were coded so that electrophysiology was performed without knowledge of which hemisphere had received 6-OHDA (ie. blind recording), and we recorded simultaneously from both hemispheres to eliminate any bias that might result from making penetrations in the two hemispheres at different times during the recording session. To make a direct correlation of catecholamine levels with plastic change we subjected tissue samples from each hemisphere to high pressure liquid chromatography (HPLC) assay of NE and dopamine (DA). The HPLC was an important addition to the experiment because it provided direct verification of the CA depletion caused by 6-OHDA treatment.

The results of our experiments are in agreement with those of Kasamatsu and Pettigrew--one week of CA depletion by infusion of 6-OHDA in 4-10 week old kittens disrupts the ocular dominance shift seen in control hemispheres. Furthermore, there appears to be a correlation of the degree of NE depletion and the loss of plasticity. Because there was considerable shift in ocular dominance in the catecholamine-depleted neonates from our previous experimental series, we speculate that under some conditions functional compensation for NE depletion can occur. The combined results imply that the level of norepinephrine is one of perhaps several factors that can influence cortical plasticity. Preliminary accounts have appeared elsewhere (Bear et al. 1982,1983).

Methods

Drug delivery and monocular deprivation

Ten kittens from our quarantined colony of wild type queens were implanted with osmotic minipumps (Table 1). Of these, we obtained complete 36 hour recordings from seven. The other three kittens died before completion of physiological recording due to respiratory complications.

Table 1 About Here

Between 4 and 10 weeks of age each kitten received ketamine-acepromazine anesthesia (25 mg/kg and 3 mg/kg respectively) and, at the same surgical session, had its left eyelid sutured closed and a micropertusion system implanted. For the microperfusion, a 23-gauge cannula was placed approximately 1/2 - 2 1/2 mm subcortically into each hemisphere through a small hole in the skull near the area centralis representation in area 17. Polyethylene tubing connected the cannulae to osmotic pumps (ALZA model 2001) subcutaneously placed at the neck. One of the pumps contained 4 mM 6-OHDA-HCl (Sigma) dissolved in saline with 0.4% ascorbate and the other contained only the vehicle solution. The solutions were infused at a rate of 1 µl/hr for one week. This procedure was used for all the kittens except K138 which received 6-OHDA in both hemispheres (Table 1). The pumps were filled and coded just prior to the surgery so that the implants were blind and at the time of physiological recording it was not known which hemisphere received the drug. The cannulae, tubing, and pumps were secured in place with a custom made skull cap held by small stainless steel screws and dental cement (Figure 1A). After return to the colony, the kittens received seven days of monocular vision on a 12 hr. light/12 hr. dark cycle. Sutures were checked daily to ensure that the deprived eye remained closed.

Figure 1 About Here

Physiological Preparation

After seven days of monocular deprivation, under ketamine-acepromazine anesthesia (25 mg/kg and 3 mg/kg respectively), each kitten received tracheal intubation and venous cannulation. For the duration of the experiment anesthesia was maintained by artificial respiration of 70% NO_2 , 28% O_2 , and 2% CO_2 while the animal was paralyzed by a continuous infusion of 12 mg/kg/hr of gallamine triethiodide (Flaxedil). The kitten was secured in a modified stereotaxic frame and the microperfusion system removed. A partial craniotomy was performed and dura was cleared in an area approximately 4 mm in diameter surrounding the previous site of cannulation for the drug delivery. During the course of the experiment EEG, body temperature, heart rate, and expired CO_2 levels were continuously monitored. Nembutal was on hand to be administered if the EEG showed signs of desynchronization.

Pupils were dilated with atropine and contact lenses were fitted to provide a slight positive correction and to prevent corneal drying. Neosynephrine was used to retract nicitating membranes. Optic discs were projected with an ophthalmoscope and marked on a tangent screen at a distance of 114 cm.

Single Unit Recording

Tungsten-in-glass microelectrodes (Levick 1972) were mounted in a dual microdrive advance which allowed simultaneous recordings to be made from similar penetrations in the two cerebral hemispheres. Each penetration passed near the area centralis representation in area 17 and down the postlateral gyrus from an initial point of entry 1/2-2 mm anterior to the previous site of cannulation. Receptive fields were plotted approximately every 100 microns of penetration. For units isolated at each depth ocular dominance was determined as well as direction selectivity, on-off properties, and speed preferences. Receptive fields were plotted using a slit of light projected on the tangent

screen. The light slit could be varied in length, width, and orientation and its position was controlled by a joystick. The ocular dominance was classified on a standard 7 point scale (Hubel and Wiesel 1962). The direction selectivity for a moving oriented bar was classified as either aspecific, immature, or specific according to criteria slightly modified from those of Fregnac and Imbert (1978). Microlesions were made at the bottom of each penetration (20 μA for 20 sec.).

Biochemistry and Histology

After completion of physiological recording (36 hours) the animals were killed by a lethal intravenous dose of potassium tartrate and perfused with saline through the ascending aorta. Tissue samples were dissected from both cortical hemispheres following several guidelines. Firstly, because the extent of catecholamine depletion is a function of the distance from the 6-OHDA infusion site, samples had to be restricted to the fraction of visual cortex which was significantly depleted (Kasamatsu et al. 1981A). Secondly, an opposing criterion was that the samples should be as large as possible to maximize the accuracy of the biochemical information from the HPLC. Finally, the specimens had to be dissected posterior to the site of electrode penetration so that electrode tracts were not damaged and could be histologically reconstructed. As a compromise based on these guidelines, each sample was a 5 mm length of the postlateral gyrus measured posterior to the site of drug infusion (Figure 1B). The tissue specimens (mean weight = 102 mg) were placed immediately on dry ice and saved for HPLC. These samples were stored overnight in a freezer at -30° C until the catecholamine purification could be performed. The remainders of the cerebral hemispheres were kept in 10% formaldehyde containing 30% sucrose for histological analysis.

Tissue catecholamines were measured by high pressure liquid chromatography with electrochemical detection. Samples were weighed,

homogenized in 0.1 M perchloric acid containing 0.1 mM EDTA, and centrifuged to remove denatured protein. Dihydroxybenzylamine, a catecholamine with a different retention time in the HPLC system than any of the naturally occurring catechols was added to the perchloric acid supernatant as a standard to monitor recovery. Catechol compounds in the supernatant were then purified by a batch aluminum oxide chromatography technique, based on a column procedure described by Anton and Sayre (1962). An aliquot of the alumina-purified sample was applied to a high pressure liquid chromatography column (Biophase ODS, 5um-Bioanalytical Systems, Inc.) which separates, in order of increasing retention time, norepinephrine, dihydroxybenzylamine, and dopamine from each other. The catechols were eluted off the column with a 0.1 M monobasic sodium phosphate buffer containing 0.13 mM sodium octyl sulfate, 0.1 mM EDTA and 4% methanol. Buffer pH was adjusted to 2.6 with phosphoric acid. The electrochemical detector oxidized the catechol compounds and the subsequent changes in current were converted into voltages and measured (Keller et al. 1976). Tissue concentrations of norepinephrine and dopamine were calculated and expressed as nanograms compound per gram tissue, wet weight.

Formalin fixed hemispheres were cut on a freezing microtome in either coronal or saggital plane at 50 microns. Sections were stained with cresyl violet acetate and electrode tracks and marker lesions were reconstructed.

Data Analysis

Of primary interest in this study was the extent to which CA depletion can limit the ocular dominance shift which normally occurs in young kittens deprived of vision through one eye. In the data analysis two quantitative measures of ocular dominance have been calculated. Binocularity (B), as defined by Pettigrew and Kasamatsu (1978), is the number of cells in ocular dominance groups 2-6 divided by the total number of visually responsive

cells. This parameter indicates whether most cells are monocularly or binocularly activated but is insensitive to the distribution of cell responses in the five ocular dominance groups which are partially binocular. We calculate the binocularity so that comparisons may be made with data from other laboratories. The other parameter which has been calculated, open eye dominance (OED), is somewhat similar to the weighted shift defined by Kasamatsu et al. (1981B). If a recording is made from the hemisphere contralateral to the non-deprived eye, a cell completely dominated by this 'open' eye would be classified in group 1. Thus we write--

$$OED = \frac{(\#\text{cells Grp 1}) + (2/3)(\#\text{cells Grp 2}) + (1/3)(\#\text{cells Grp 3})}{\text{Total}\ \#\ \text{cells}}$$

Similarly, if the recording is ipsilateral to the open eye, groups 1, 2, 3 are replaced by groups 7, 6, 5 in this equation. An OED value of zero would indicate that no cells were driven predominantly by the non-deprived eye whereas positive values would indicate the degree of shift toward entirely open eye monocular cells. Because the OED does not weight cells dominated by the deprived eye it avoids an ambiguity present in other indices of ocular dominance shift--if cells in all seven ocular dominance groups are counted, the value of a parameter doesn't distinguish between a few cells dominated by the open eye and many cells slightly dominated by the closed eye. We therefore feel the OED parameter is more strictly a quantification of the degree of shift in ocular dominance because it only counts cells with responses dominated by the non-deprived eye and it is weighted in a straightforward manner so that highly monocular cells are most significant.

In the statistical analysis of the binocularity, OED, and catecholamine

levels a Wilcoxon-Mann-Whitney rank sum test has been used. This non-parametric test yields information on levels of confidence similar to the common Student's t test but avoids assumptions about the normality of the parameter distributions.

Results

Figure 2 illustrates the intracortical effect of the cannulation for drug delivery. In a sphere roughly 1.5 mm in radius centered on the cannula tip there was nonspecific damage of all tissue in both drug- and vehicle-treated hemispheres. The shaded area in figure 2 indicates the approximate region (6 mm radius) where selective damage of catecholaminergic terminals should have occurred in the drugged hemisphere based on studies of 6-OHDA spread by Kasamatsu et al. (1981A). In particular, within this region NE levels should be less than 50% of control (according to Kasamatsu et al. 1981A, NE was approximately 35% of control 6 mm from the cannula site). For successful electrophysiological recording it was imperative that the electrode passed anterior to the nonspecific destruction into this selectively depleted zone. In fact, figure 2 illustrates a case (K142) in which only 14 single units were isolated during physiological recording. The histological reconstruction revealed that the electrode was oriented at too acute an angle and stayed in the selectively depleted region for only a short time before entering damaged tissue. Generally, during recording, if we suspected that the electrode had entered the zone of nonspecific damage a new penetration was made at a more obtuse angle.

Figure 2 About Here

Following monocular deprivation, single unit recordings from control hemispheres were largely dominated by the non-deprived eye whereas responses from drug-treated hemispheres were significantly more binocular. The ocular dominance histograms for K136 are examples of this shown in figure 3. In this 65 day old animal the control hemisphere gave B = 0.32 and OED = 0.71 whereas the drug treated hemisphere gave B = 0.68 and OED = 0.28. These numbers quantify what is obvious from the histograms--cell responses in the 6-OHDA-treated cortex were both more binocular and less shifted toward the non-deprived eye compared to cells in the cortex infused only with vehicle solution. Furthermore, figure 4 shows that this difference was not a result

Figure 3 About Here

Figure 4 About Here

of disparate sampling frequencies or sampling depths. This figure also demonstrates that receptive field eccentricities were similar along penetrations in the two hemispheres, ruling out the possibility of a large topographical sampling bias. The HPLC analysis of cortical samples from K136 yielded catecholamine levels which were lower in the 6-OHDA treated hemisphere than in the vehicle treated hemisphere (Table 2). In the tissue pertused with 6-OHDA, NE was reduced to only 12% of the control level whereas DA was 93% of control (Figure 5). There were no differences between hemispheres regarding on-off properties or speed preferences for a moving oriented slit of light. Cells in the drugged hemisphere had slightly broader direction selectivities, but this was not a consistent distinction in the other experimental animals. In summary, K136 demonstrates that a significant depletion of norepinephrine accompanies a decrease in ocular dominance plasticity following cortical 6-OHDA infusion but, with this paradigm, other receptive field properties are not altered.

Table 2 About Here

Figure 5 About Here

The results from the seven successfully recorded animals support the conclusions drawn from the K136 example. The normalized composite histograms for all kittens are shown in figure 6. The 6-OHDA treatment had a marked effect on the tendency of cells to be dominated by the non-deprived eye following monocular rearing. In all cases the cells in drug-treated tissue were far less shifted in ocular dominance, as if the cortical plasticity had been disrupted. The binocularity and open-eye dominance for all kittens are listed in table 2--the averages for all control hemispheres are B = 0.54 $\pm$ 0.07 ($\bar{x} \pm$ sem) and OED = 0.64 $\pm$ 0.04 compared to B = 0.75 $\pm$ 0.04 and OED = 0.30 $\pm$ 0.06 for drug-treated hemispheres. Using the rank sum test, the 6-OHDA- and vehicle-treated populations are different at the confidence level $p < 0.0025$. This level of significance is reached by comparison of either binocularity or OED parameters. It is reassuring that the populations are clearly distinct irrespective of the particular parameter used to characterize the ocular dominance distributions. A search was made for other receptive

Figure 6 About Here

field properties which were affected by the 6-OHDA treatment but direction selectivity, on-off properties, and speed preferences showed no consistent difference between drug and control tissue. Behaviorally it was found that all animals successfully depleted of catecholamines were listless and gained little or no weight during the week of monocular deprivation and 6-OHDA infusion (Table 1).

Because we attempt to relate biochemical and physiological changes in cortex, the HPLC confirmation of catecholamine depletion is of critical importance. An example will illustrate this point. Animal K140 had a 6-OHDA filled minipump connected to one cortical hemisphere and a vehicle filled pump connected to the other hemisphere, concurrent with seven days of monocular vision. Subsequent single unit recording yielded ocular dominance histograms for <u>both</u> hemispheres which were shifted toward the non-deprived eye. The biochemical analysis which followed revealed that, in fact, <u>neither</u> hemisphere had been successfully depleted of catecholamines. For this reason only the control hemisphere is listed in table 2. The cause of this negative result was probably an obstruction in the tubing of the 6-OHDA delivery system. The important point is that the data could easily have been misinterpreted if the HPLC had not been used to verify the effectiveness of the 6-OHDA treatment.

In all cases the lack of ocular dominance shift in hemispheres successfully infused with 6-OHDA was accompanied by lowered levels of NE ($\bar{x} \pm$ sem = 21% $\pm$ 6%) and DA (54% $\pm$ 10%) relative to the control hemispheres in the same animal (Table 2, Figure 5). Based on absolute levels of norepinephrine the 6-OHDA-treated and control populations are different at the confidence level $p < 0.05$. The dopamine levels were not as consistently different ($p < 0.1$). Although there was a large variation in absolute neurotransmitter levels (Table 2), figure 5 shows that the percentages of depletion relative to same-animal controls were more consistent. The reason for the variations in catecholamine levels is not known but, we emphasize that the biochemical

analysis always distinguished the two hemispheres of each kitten because NE was always depleted by 50% or more in the 6-OHDA-treated cortex. This distinction is significant because in our experience, the interhemispheric variability in CA levels of untreated kittens is quite low (< 5%), regardless of the interindividual variation. In the present study we conclude that the difference in NE levels between 6-OHDA-treated and control cortices is a valid distinction in light of the statistics. Furthermore, the data suggest that with our particular experimental paradigm there is a positive correlation between the percent of NE in the 6-OHDA treated cortex (relative to control tissue in the same animal) and the ability of cells to shift in ocular dominance (Figure 7). Caution is justified in accepting this relationship based on the data from only 7 kittens, but the high correlation coefficient (r = 0.87) certainly warrants further investigation. There was no indication of a correlation of dopamine depletion and ocularity.

Figure 7 About Here

Discussion

Our data confirm the observation that one week of intracortical infusion of 6-OHDA concurrent with monocular deprivation disrupts ocular dominance plasticity in area 17. Control hemispheres displayed an ocular dominance shift to the open eye but cortical hemispheres perfused with 6-OHDA failed to shift. Furthermore, the drug treated tissues had norepinephrine levels significantly less than control ($\bar{x} \pm$ sem = 21% $\pm$ 6%) and dopamine levels somewhat less than control (54% $\pm$ 10%) as determined by high pressure liquid chromatography. The effect of 6-OHDA on other neurotransmitters was not examined but there is strong evidence that 6-OHDA administered in this manner destroys mainly catecholaminergic nerve terminals (Breese and Traylor 1970; Kasamatsu et al. 1981A; Ungerstedt 1968; Uretsky and Iversen 1970).

Although cortical infusion of 6-OHDA clearly decreases plasticity,

considerably more evidence has been needed to characterize the role that NE plays in regulating critical period plasticity. In fact, based solely on the data from our laboratory one cannot rule out the possibility that loss of plasticity is caused directly by 6-OHDA and not by loss of catecholamines. However, it has been shown that 6-OHDA treatment prevents an ocular dominance shift even when the treatment is stopped a day before monocular deprivation begins (Kasamatsu and Pettigrew 1979). This is the strongest evidence that 6-OHDA exerts its effect by destruction of catecholaminergic fibers rather than by direct toxicity. Because recent evidence indicates that distinct DA and NE systems project to visual cortex (Itakura et al. 1981; Tork et al. 1979; Tork and Turner 1981) and that both are affected by 6-OHDA (Breese and Traylor 1970; Ungerstedt 1968; Uretsky and Iversen 1970), experiments have been performed which have probed the relative importance of each system for plasticity: firstly, our HPLC analysis has shown that administration of 6-OHDA depletes NE significantly more than DA. Secondly, Kasamatsu and colleagues (Pettigrew and Kasamatsu 1978; Kasamatsu et al. 1979) have restored some plasticity to area 17 of 6-OHDA-treated animals by microperfusion of NE into the visual cortex (ie. replacement of depleted NE). This is also compelling evidence that loss of plasticity is caused by depletion of NE and not by 6-OHDA toxicity. And finally, our data indicate that, under the conditions of this experiment, depletion of cortical NE (relative to same-animal control tissue) is accompanied by a roughly proportional decrease in the ocular dominance shift following monocular deprivation, as measured by the open-eye dominance parameter. There was no simple relationship between the DA depletion and the shift in ocular dominance. Collectively these experiments suggest that NE is the catecholamine that governs plasticity.

Although a decrease in ocular dominance plasticity was the only consistent effect of cortically infused 6-OHDA which we observed, it is likely that 6-OHDA can influence plasticity for other receptive field

properties. Daw and coworkers have given 6-OHDA to kittens reared in an environment continually moving in one direction (Daw et al. 1981). Subsequent electrophysiological recording revealed that visually activated cells in control animals responded optimally to movement in the direction of the artificial environment but cells in drug-treated kittens showed no such direction preference. Therefore, under the proper rearing conditions the 6-OHDA does seem to limit neuronal plasticity affecting direction selectivity. Our results together with this finding might lead one to speculate that depletion of NE causes a general disruption of all receptive field plasticity.

However, cortical plasticity cannot simply be determined by NE levels because it has been shown that over 95% depletion of NE in neonates has no significant effect on the potential for plastic changes in ocular dominance (Bear et al. 1982). In that case the 6-OHDA treatment differed from the current study both in timing and duration. Evidently, when NE is systemically depleted for a month from birth the developing nervous system is able to compensate for the loss. Receptor supersensitivity is one mechanism that compensates for chronic denervation under some circumstances (Sporn et al. 1977), but available evidence suggests that this response alone is incapable of making up for 90% depletion of cortical NE (Harik et al. 1981). Other forms of compensation, such as sprouting or degeneration of fibers from other transmitter systems are possible and indeed, Harik et al. have observed a compensation phenomenon in rat cerebral cortex independent of receptor supersensitivity. At any rate, plasticity in the absence of NE doesn't belie the importance of norepinephrine, instead it may indicate that the interplay of several factors determines cortical plasticity and compensation can sometimes be made for the loss of one of these elements.

It has recently been shown by Singer that cortical plasticity is also lost following destruction of certain medial thalamic nuclei (Singer 1982). The lesion seems to disrupt the animal's normal faculties of selective

attention which may be required for experience dependent modification of visual cortex. Our 6-OHDA-treated animals may also have shown an attentional deficit. All animals which were successfully depleted of catecholamines were listless during the week of 6-OHDA treatment. This inactivity appeared to even prevent the kittens from walking across their cage in order to eat, which caused most to lose weight. This behavioral observation is consistent with a remark made by Kasamatsu and Pettigrew (1979), concerning kittens given intraventricular injections of 6-OHDA. Their kittens appeared to be unable to learn the location of food and sometimes had to be hand fed even though they displayed a healthy appetite. It should be noted however, that Kasamatsu and Pettigrew did not report this behavioral effect in their animals that received 6-OHDA from osmotic pumps (Kasamatsu et al. 1979). The effect we observed may have been produced by the action of 6-OHDA on subcortical structures. However, since it is not known what the role of NE is in normal cat visual cortex, it may be prudent to consider the possibility that the loss of plasticity associated with deficits in selective attention, as observed by Singer, is not entirely independent of the loss of plasticity caused by 6-OHDA treatment.

On balance the evidence indicates that in kittens 4 weeks to 10 weeks of age, norepinephrine in area 17 is usually of critical importance for cortical modifiability. NE may act as a neurohumor during this time by 'modulating' the level of a second messenger such as cAMP, thus affecting protein synthesis and enhancing any synaptic modification (Kasamatsu et al. 1979). Whether or not this is how NE governs plasticity, it is apparent that other factors also affect experience dependent modification of visual cortex. It is likely that the elucidation of the mechanism by which NE effects plasticity will also explain when and how compensation for lost NE can occur.

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References

Anton AH, Sayre DF (1962) A study of the factors affecting the aluminum-trihydroxy-indole procedure for the analysis of catecholamines. J Pharmacol Exp Ther 138: 360-375.

Bear MF, Daniels JD (in press) The plastic response to monocular deprivation persists in kitten visual cortex after chronic depletion of norepinephrine. J Neurosci.

Bear MF, Paradiso MA, Schwartz M, Nelson SB, Carnes KM, Daniels JD (in press) Two methods of catecholamine depletion in kitten visual cortex yield different effects on plasticity. Nature.

Bear MF, Paradiso MA, Daniels JD (1982) Visual cortical plasticity: deficit after acute, but not chronic, noradrenergic denervation with 6-hydroxydopamine. Soc Neurosci Abstr 8: 4.

Breese GR, Traylor TD (1970) Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: evidence for selective degeneration of catecholamine neurons. J Pharm Exp Ther 174: 413-420.

Clark DW, Laverty R, Phelan EL (1972) Long-lasting peripheral and central effects of 6-hydroxydopamine in rats. Brit J Pharmacol 44: 233-243.

Crow TJ (1968) Cortical synapses and reinforcement: a hypothesis. Nature 219: 736-737.

Daw NW, Rader RK, Robertson TW (1981) Effect of 6-hydroxydopamine on plasticity of direction selective cells in visual cortex. Soc Neurosci Abstr 7: 673.

Fregnac Y, Imbert M (1978) Early development of visual cortical cells in normal and dark-reared kittens: relationship between orientation selectivity and ocular dominance. J Physiol (London) 278: 27-44.

Harik SJ, Duckrow RB, LaManna JC, Rosenthal M, Sharma VK, Banerjee SP (1981) Cerebral compensation for chronic noradrenergic denervation induced by locus coeruleus lesion: recovery of receptor binding, isoproterenol-induced adenylate cyclase activity, and oxidative metabolism. J Neurosci 1: 641-649.

Hubel DH, Wiesel TN (1962) Receptive fields, binocular interactions and functional architecture in the cat's visual cortex. J Physiol 160: 106-154.

Itakura T, Kasamatsu T, Pettigrew JD (1981) Norepinephrine-containing terminals in kitten visual cortex: laminar distribution and ultrastructure. Neurosci 6: 159-175.

Kasamatsu T, Pettigrew JD (1976) Depletion of brain catecholamines: failure of ocular dominance shift after monocular occlusion in kittens. Science 194: 206-209.

Kasamatsu T, Pettigrew JD (1979) Preservation of binocularity after monocular deprivation in the striate cortex of kittens treated with 6-hydroxydopamine. J Comp Neurol 185: 139-162.

Kasamatsu T, Pettigrew JD, Ary M (1979) Restoration of visual cortical plasticity by local microperfusion of norepinephrine. J Comp Neurol 185: 163-182.

Kasamatsu T, Itakura T, Jonsson G (1981A) Intracortical spread of exogenous catecholamines: effective concentration for modifying cortical plasticity. J Pharmacol Exp Ther 217: 841-850.

Kasamatsu T, Pettigrew JD, Ary M (1981B) Cortical recovery from effects of monocular deprivation: acceleration with norepinephrine and suppression with 6-hydroxydopamine. J Neurophysiol 45: 272-284.

Keller R, Oke A, Mefford I, Adams RN (1976) Liquid chromatographic analysis of catecholamines: routine assay for regional brain mapping. Life Sci 19: 995-1004.

Kety SS (1970) The biogenic amines in the central nervous system: their possible roles in arousal, emotion, and learning. In: Schmitt FO (ed), The Neurosciences, Second Study Program. Rockefeller University Press, New York, pp 324-336.

Levick WR (1972) Another tungsten microelectrode. Med Biol Engin 10: 510-515.

Pettigrew JD, Kasamatsu T (1978) Local perfusion of noradrenaline maintains visual cortical plasticity. Nature 271: 761-763.

Sachs C (1973) Development of the blood-brain barrier for 6-hydroxydopamine. J Neurochem 20: 1753-1760.

Sachs S, Jonsson G (1975) Effects of 6-hydroxydopamine on central noradrenaline neurons during ontogeny. Br Res 99: 277-291.

Singer W (1982) Central core control of developmental plasticity in the kitten visual cortex: I. Diencephalic lesions. Exp Br Res 47: 209-222.

Sporn JR, Wolfe BB, Harden TR, Kendall T, Molinoff PB (1977) Supersensitivity in rat cerebral cortex: Pre- and post-synaptic effects of 6-hydroxydopamine at noradrenergic synapses. Mol Pharmacol 13: 1170-1180.

Tork I, Levanthal AG, Stone J (1979) Brain stem afferents to visual cortical areas 17, 18 and 19 in the cat, demonstrated by horseradish peroxidase. Neurosci Lett 11: 247-252.

Tork I, Turner S (1981) Histochemical evidence for a catecholaminergic (presumably dopaminergic) projection from the ventral mesencephalic tegmentum to visual cortex in the cat. Neurosci Lett 24: 215-219.

Ungerstedt U (1968) 6-hydroxydopamine induced degeneration of central monoamine neurons. Eur J Pharmacol 5: 107-110.

Uretsky NJ, Iversen LL (1970) Effects of 6-hydroxydopamine on catecholamine containing neurons in the rat brain. J Neurochem 17: 269-278.

Table 1 Animal History

Animal	Age(days) at Implant	Age(days) at Physiol.	Weight(gm) at Implant	Weight(gm) at Physiol.	Drug Treatment Left Hem.	Drug Treatment Right Hem.
K134	44	51	480	532	6-OHDA	Vehicle
K135	51	58	756	672	6-OHDA	Vehicle
K136	58	65	728	672	Vehicle	6-OHDA
K137	71	79	784	754	6-OHDA	Vehicle
K138	31	38	504	454	6-OHDA	6-OHDA
K139	37	44[a]	454	a	6-OHDA	Vehicle
K140	45	52	672	644	Vehicle	6-OHDA[b]
K141	52	59[a]	756	756	Vehicle	6-OHDA
K142	27	34[a]	454	532	Vehicle	6-OHDA
K143	40	47	560	560	Vehicle	6-OHDA

a indicates an animal that died before completion of full 36 hr recording session.

b As described in the Results, the right hemisphere in K140 was not successfully depleted of catecholamines.

Table 2 Summary of Ocular Dominance and Biochemical Data

Control Hemispheres

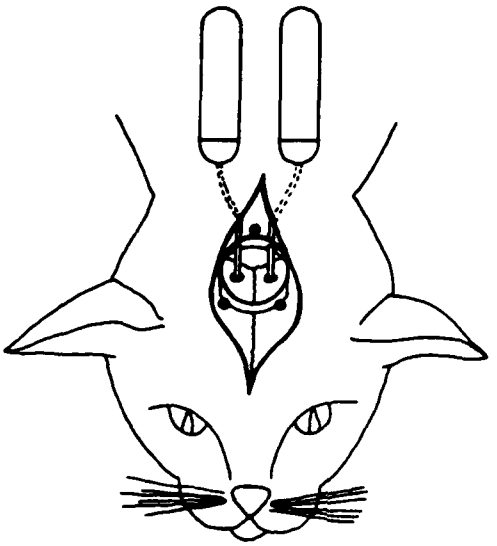
Animal	B	OED	NE (ng/gm)	DA (ng/gm)
K134	0.56	0.64	135	110
K135	0.36	0.77	406	163
K136	0.32	0.71	710	71
K137	0.69	0.65	189	228
K140[a]	0.70	0.56	303	118
K143	0.62	0.49	1169	325
$\bar{x}$ ± sem =	0.54 ± 0.07	0.64 ± 0.04	485 ± 160	169 ± 38

6-OHDA Treated Hemispheres

Animal	B	OED	NE (ng/gm)	DA (ng/gm)
K134	0.69	0.38	17	63
K135	0.65	0.55	207	109
K136	0.68	0.28	87	66
K137	0.86	0.37	71	93
K138l	0.78	0.09	28	<35
K138r	0.70	0.33	48	46
K143	0.92	0.13	261	236
$\bar{x}$ ± sem=	0.75 ± 0.04	0.30 ± 0.06	103 ± 36	<93 ± 26

a The right hemisphere data for K140 are not tabulated because this was not strictly a control hemisphere and also was not depleted of NE to our criterion level of 50% (see Results).

A



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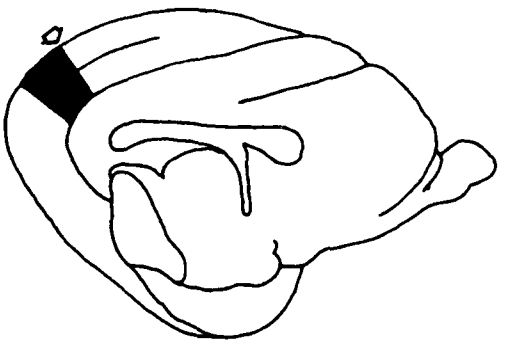

Figure 1

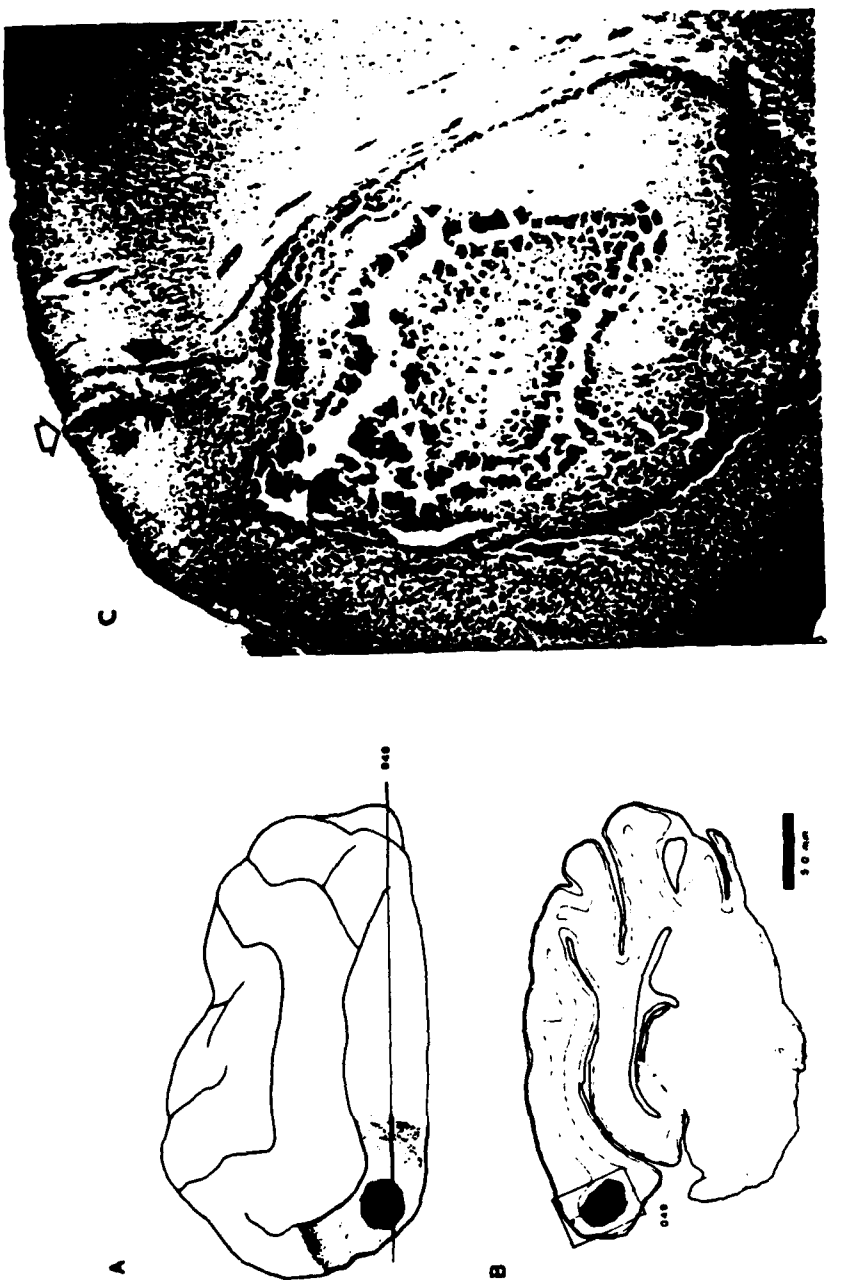

Figure 2

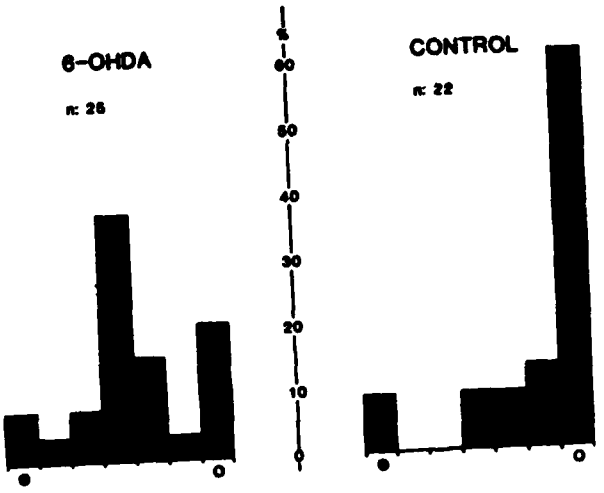

Figure 3

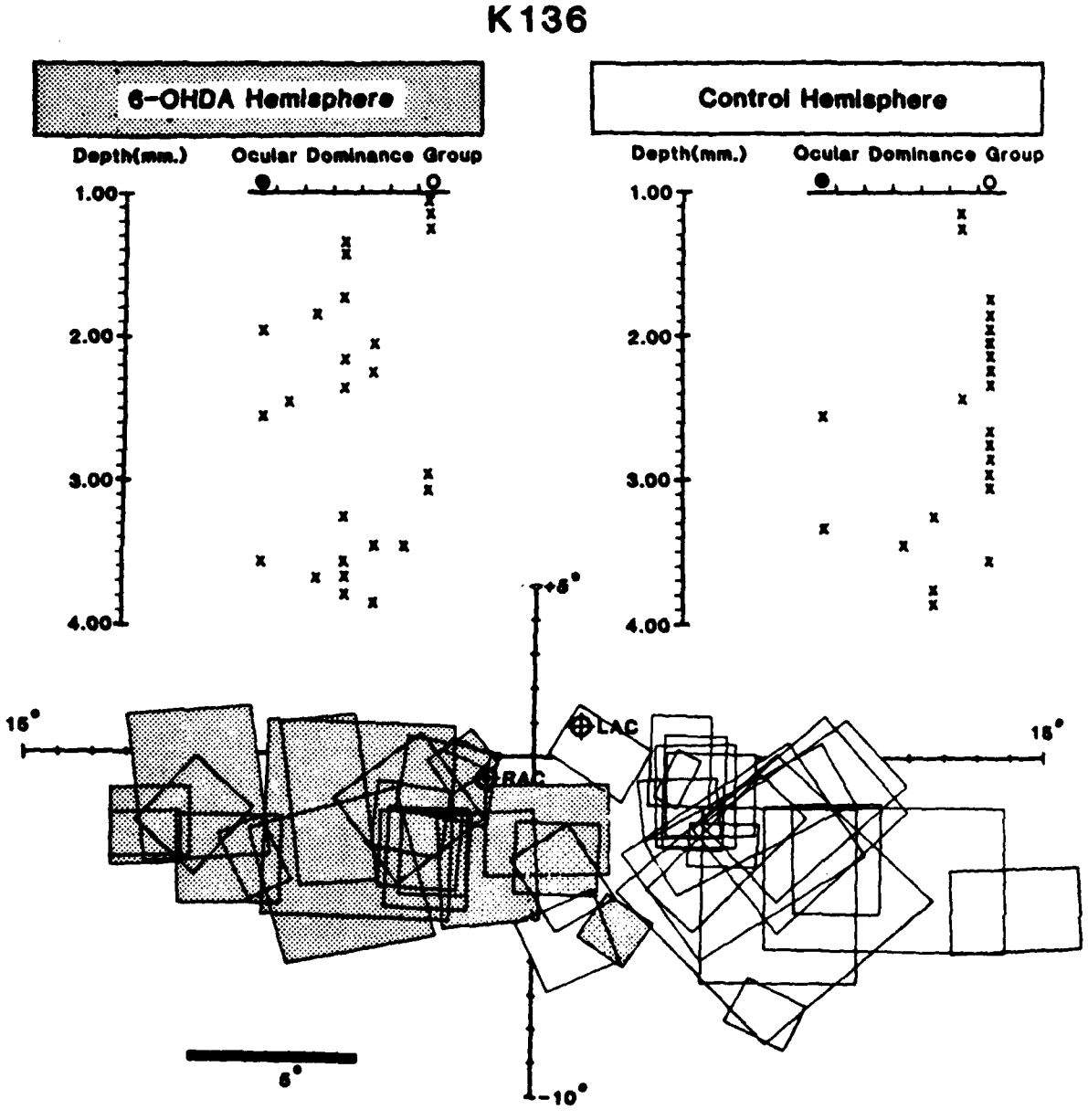
K136
6-OHDA Hemisphere
Control Hemisphere
Depth(mm.)
Ocular Dominance Group
1.00
2.00
3.00
4.00
+5°
16°
18°
LAC
RAC
5°
-10°

Figure 4

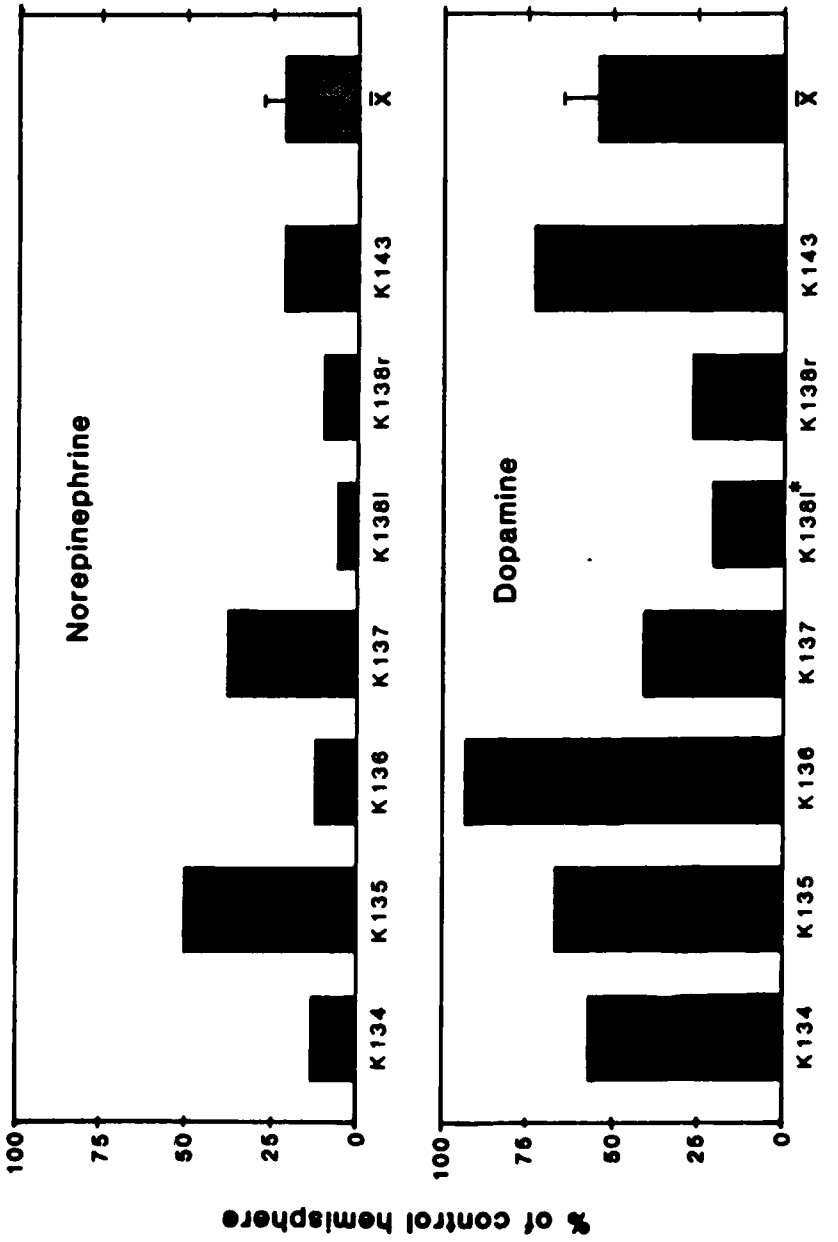
Norepinephrine
Dopamine
% of control hemisphere
100
75
50
25
0
K134
K135
K136
K137
K138l
K138r
K143
X̄
K138l*

Figure 5

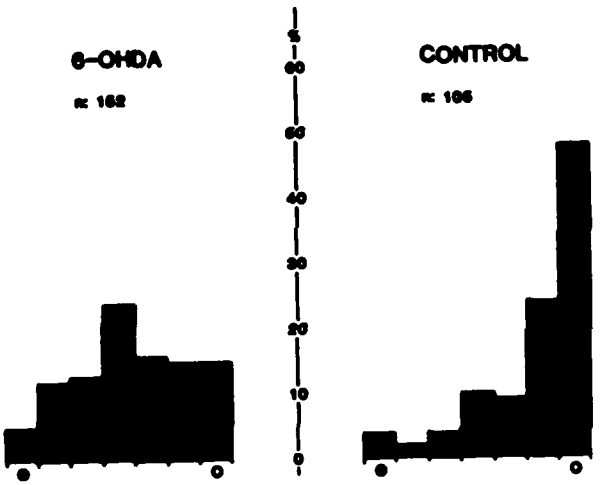

Figure 6

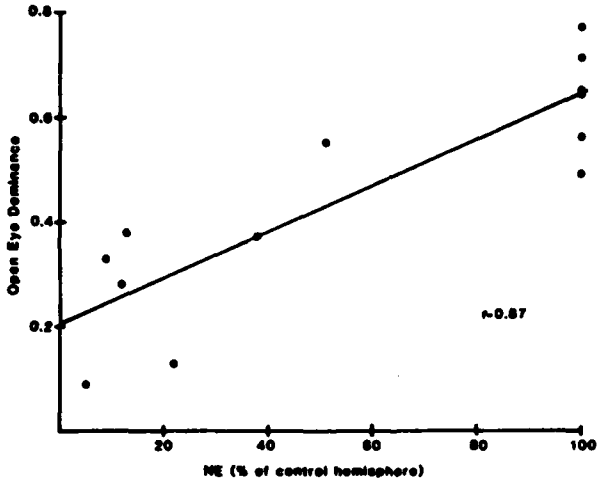
0.8
0.6
0.4
0.2
Open Eye Dominance
r=0.67
20
40
60
80
100
NE (% of control hemisphere)

Figure 7

Figure Captions

1) (A) Detail of minipump-cannula implant. Osmotic pumps were connected by polyethylene tubing to 23-gauge cannulae placed 1/2-2 1/2 mm subcortically in each hemisphere near the areae centralis representations in area 17. A skull cap of PVC tubing secured the pump system and was held in place by steel screws and dental cement. The cannulae were fixed in place by dental cement which filled the PVC chamber. (B) Saggital section showing location of biochemical sample. After electrophysiological recording a 5 mm length of each postlateral gyrus (shaded), measured posterior to the site of cannulation (arrow), was removed for HPLC analysis.

2) Nonspecific damage caused by cortical cannulation. In both 6-OHDA and vehicle-treated hemispheres nonspecific tissue destruction occurred in a region approximately 1.5 mm in radius centered on the cannula tip. (A) is a dorsal reconstruction of the area of nonspecific destruction. NE levels should have been reduced to less than 50% of control within the shaded region (Kasamatsu et al. report levels approximately 35% of control 6 mm away from the cannula). (B) is a parasagittal section of the damaged cortex and serves to locate the section enlarged in the photomicrograph (C). The solid arrow indicates an electrode tract and the open arrow points to the site of cannulation.

3) Normalized ocular dominance histograms for 6-OHDA-treated and control hemispheres in animal K136 following 7 days of monocular deprivation. Ocular dominance is classified into 7 groups (Hubel and Wiesel 1962) where the closed circle is under the deprived eye group and the open circle is under the non-deprived eye group. In the vehicle-treated hemisphere 32% of the cells were binocular (OED = 0.71) compared to 68% (OED = 0.28) in the 6-OHDA treated hemisphere. See Methods for definition of OED (open eye dominance).

4) The table at the top of the figure demonstrates that the difference in ocular dominance distributions between drug-treated and control hemispheres for K136 was not caused by a bias in sampling frequency or depth. The lower figure is a plot of receptive fields for 6-OHDA treated (shaded) and control hemispheres. The eccentricities of receptive fields reveal no significant topographical sampling bias.

5) Catecholamine levels in hemispheres treated with 6-OHDA for one week. Each percentage is the CA level in the drugged hemisphere relative to the control hemisphere in the same animal. K138 received 6-OHDA in both hemispheres and its %NE and %DA are calculated relative to the mean NE and DA levels for all control hemispheres. In drugged hemispheres the mean NE level was 21% of control and the mean DA level was 54% of control. Based on absolute levels of norepinephrine the 6-OHDA-treated and control populations are different at the confidence level $p < 0.05$.

6) Ocular dominance distribution summarized for 7 kittens listed in table 2. In 6-OHDA treated hemispheres 75% of the cells were binocular (OED = 0.30 ± 0.06) whereas only 54% were binocular (OED = 0.64 ± 0.04) in control hemispheres. This difference is significant at the confidence level $p = 0.0025$. Conventions are as in figure 3.

7) Open eye dominance (OED) is plotted as a function of %NE level in the 6-OHDA treated hemisphere. The %NE is for drugged hemispheres relative to same animal control hemispheres as shown in figure 5. The line is a least squares fit to the data. The data gives a Pearson correlation coefficient of $r = 0.87$.

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